




# Draft Genomic Sequences of Four *Pseudomonas* spp. and a *Xanthomonas* sp. from Cranberry Stem Galls

Brian Graham,<sup>b</sup> Tawny Castañeda,<sup>a</sup> Alisha Harrison,<sup>a</sup>  Scott Soby<sup>a,c</sup>

<sup>a</sup>Biomedical Sciences Program, College of Graduate Studies, Midwestern University, Glendale, Arizona, USA

<sup>b</sup>Arizona College of Osteopathic Medicine, Midwestern University, Glendale, Arizona, USA

<sup>c</sup>College of Veterinary Medicine, Midwestern University, Glendale, Arizona, USA

**ABSTRACT** Four *Pseudomonas* spp. and *Xanthomonas arboricola* were isolated from cranberry stem galls in Carver, MA, and taxonomically assigned at the genus level based on the 16S rRNA sequence and phenotypes. *X. arboricola* had not been associated previously with cranberry stem galls or any cranberry disease.

Stem galls on cranberry plants (*Vaccinium macrocarpon* Ait.) result from hyperplasia and hypertrophy in response to the production of phytohormones by bacteria that have invaded stem tissue, usually following mechanical or frost injury to the epidermis (1). Galls are relatively common in commercial cranberry operations in regions with especially cold winters and are likely the result of mixed infections, of which some members produce indole-3-acetic acid (IAA) (2). Although infrequently observed in Massachusetts, galls can girdle the stem, resulting in the death of meristems and fruit-producing organs. Bacteria were isolated from multiple stem galls on several plants in a commercial cranberry bog in Carver, MA, following the severe 2015 winter by spreading surface-sterilized gall tissue on nonselective media. Five of the isolates were transferred to King's medium B (KMB) agar containing 50  $\mu\text{g} \cdot \text{mL}^{-1}$  each of cycloheximide and ampicillin, incubated at 26°C for 24 to 48 h, colony purified 3 times, and stored at  $-80^{\circ}\text{C}$  in 34% glycerol. Four isolates were placed in the genus *Pseudomonas* and one in *Xanthomonas* by 16S rRNA gene sequences amplified with 27F and 1525R primers, using BLAST (3) within the NCBI nucleotide database. Isolates from frozen storage were recovered on KMB agar, and then populations were inoculated into overnight KMB broth cultures for genomic DNA isolation with a DNeasy blood and tissue kit (Qiagen). Illumina-compatible genomic DNA (gDNA) libraries were generated with a Kapa Biosystem Hyperplus library preparation kit (KK8514). DNA was enzymatically sheared to approximately 500-bp fragments, end repaired, and A-tailed. Illumina-compatible adapters with unique indexes (Integrated DNA Technologies [IDT] number 00989130v2) were individually ligated to each sample, cleaned using pure beads (Kapa Biosciences; KK8002), and amplified with a HiFi enzyme (KK2502). Each library was analyzed for fragment size (Agilent TapeStation) and quantified by quantitative PCR (qPCR) (Kapa library quantification kit, KK4835; Thermo Fisher Scientific, QuantStudio 5) before multiplex pooling and Illumina MiSeq sequencing on a 2  $\times$  250-bp flow cell. The assembly of raw reads was done by Unicycler v0.4.8 (4) and polished with Pilon v1.23 (5) within the PATRIC Comprehensive Genome Analysis pipeline v3.6.12 using default settings (<http://patricbrc.org>) (6) (Table 1), which includes Trim Galore v0.4.0 ([https://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) (7) for adapter trimming and quality control. Genome sequences were annotated using RASTtk (8) as part of the PATRIC pipeline. Using the Type (Strain) Genome Server (9), isolates were placed within *Pseudomonas syringae* (MWU16-30316), *Pseudomonas putida* (MWU16-30317), or *Pseudomonas fluorescens* subgroups *P. fluorescens* (MWU16-30323) and *Pseudomonas korensis* (MWU16-30322). MWU16-30325 is most closely related to *X. arboricola* pv. *pruni*. RAST annotation indicates that MWU16-30322, MWU16-30316, and MWU16-30325 contain the

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Address correspondence to Scott Soby, ssobyx@midwestern.edu.

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TABLE 1 Data summary

Isolate	Assigned taxon	Genome size (bp)	No. of contigs	N <sub>50</sub> contig size (bp)	Coverage (x)	G+C content (%)	Total length of reads (bp)	No. of reads	BioSample accession no.	GenBank accession no.	SRA accession no.	No. of coding sequences	No. of tRNA/rRNA genes
MWU16-30322 (Erika 2)	<i>Pseudomonas</i> nov. sp.	6,193,752	37	428,342	362	60.41	2,241,274,727	4,830,691	<a href="#">SAMN21542436</a>	<a href="#">JAIWYL0000000000</a>	<a href="#">SRX12391655</a>	5,709	63/2
MWU16-30316 (Erika 3)	<i>Pseudomonas</i> nov. sp.	5,994,666	25	858,102	301	59.17	1,806,931,759	3,033,833	<a href="#">SAMN21542437</a>	<a href="#">JAIZAZ0000000000</a>	<a href="#">SRX12300885</a>	5,350	55/4
MWU16-30323 (Erika 4)	<i>Pseudomonas</i> nov. sp.	6,669,642	112	205,587	164	60.86	1,090,791,137	2,442,946	<a href="#">SAMN21542439</a>	<a href="#">JAIWYM0000000000</a>	<a href="#">SRX12391656</a>	6,149	46/2
MWU16-30317 (Erika 5)	<i>Pseudomonas</i> nov. sp.	6,473,724	37	904,938	1668	61.39	2,744,746,383	5,223,120	<a href="#">SAMN21542440</a>	<a href="#">JAIWJA0000000000</a>	<a href="#">SRX12300886</a>	5,888	58/3
MWU16-30325 (Erika 7)	<i>Xanthomonas</i> sp.	4,811,759	13	1,303,235	288	65.89	1,384,143,382	2,899,649	<a href="#">SAMN21542459</a>	<a href="#">JAIWYN0000000000</a>	<a href="#">SRX12391657</a>	4,293	51/3

gene (*ina*) required for the ice nucleation protein that is associated with frost damage (10, 11) and that *Xanthomonas* sp. MWU16-30325 in addition contains genes for assembly and translocation of xanthan (12).

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank BioProject [PRJNA765055](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA765055) under the accession numbers [JAIWYL000000000](https://www.ncbi.nlm.nih.gov/nuclot/JAIWYL000000000) (MWU16-30322), [JAIAZ000000000](https://www.ncbi.nlm.nih.gov/nuclot/JAIAZ000000000) (MWU16-30316), [JAIWYM000000000](https://www.ncbi.nlm.nih.gov/nuclot/JAIWYM000000000) (MWU16-30323), [JAIWJA000000000](https://www.ncbi.nlm.nih.gov/nuclot/JAIWJA000000000) (MWU16-30317), and [JAIWYN000000000](https://www.ncbi.nlm.nih.gov/nuclot/JAIWYN000000000) (MWU16-30325). The versions described in this paper are the first versions, [JAIWYL000000000.1](https://www.ncbi.nlm.nih.gov/nuclot/JAIWYL000000000.1), [JAIAZ000000000.1](https://www.ncbi.nlm.nih.gov/nuclot/JAIAZ000000000.1), [JAIWYM000000000.1](https://www.ncbi.nlm.nih.gov/nuclot/JAIWYM000000000.1), [JAIWJA000000000.1](https://www.ncbi.nlm.nih.gov/nuclot/JAIWJA000000000.1), and [JAIWYN000000000.1](https://www.ncbi.nlm.nih.gov/nuclot/JAIWYN000000000.1), respectively. Links to SRA accessions are provided in Table 1. RASTtk annotations are available under open license at Zenodo (<https://zenodo.org/record/5949069#.Yf1TcfnMKU1>).

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## REFERENCES

1. Vasanthakumar A, McManus PS. 2004. Indole-3-acetic acid-producing bacteria are associated with cranberry stem gall. *Phytopathology* 94:1164–1171. <https://doi.org/10.1094/PHYTO.2004.94.11.1164>.
2. Best VM, Vasanthakumar A, McManus PS. 2004. Anatomy of cranberry stem gall and localization of bacteria in galls. *Phytopathology* 94:1172–1177. <https://doi.org/10.1094/PHYTO.2004.94.11.1172>.
3. Auch AF, Henz S, Holland B, Göker M. 2006. Genome BLAST distance phylogenies inferred from whole plastid and whole mitochondrion genome sequences. *BMC Bioinformatics* 7:350. <https://doi.org/10.1186/1471-2105-7-350>.
4. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
5. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
6. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. *Nucleic Acids Res* 45:D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
7. Bush SJ. 2020. Read trimming has minimal effect on bacterial SNP-calling accuracy. *Microbial Genomics* 6:mgen000434. <https://doi.org/10.1099/mgen.0.000434>.
8. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <https://doi.org/10.1038/srep08365>.
9. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 10:2182. <https://doi.org/10.1038/s41467-019-10210-3>.
10. Workmaster BAA, Palta JP, Wisniewski M. 1999. Ice nucleation and propagation in cranberry uprights and fruit using infrared video thermography. *J Am Soc Hortic Sci* 124:619–625. <https://doi.org/10.21273/JASHS.124.6.619>.
11. Gurian-Sherman D, Lindow SE. 1993. Bacterial ice nucleation: significance and molecular-basis. *FASEB J* 7:1338–1343. <https://doi.org/10.1096/fasebj.7.14.8224607>.
12. Galván EM, Ielmini MV, Patel YN, Bianco MI, Franceschini EA, Schneider JC, Ielpi L. 2013. Xanthan chain length is modulated by increasing the availability of the polysaccharide copolymerase protein GumC and the outer membrane polysaccharide export protein GumB. *Glycobiology* 23:259–272. <https://doi.org/10.1093/glycob/cws146>.